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Supporting Information

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Engineering the Structural Properties of DNA Blockcopolymer Micelles by Molecular Recognition

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Content

I. Material Preparation

The preparation of ss DNA-*b*-PPO diblock copolymers, and the formation of micelles were carried out as described previously.[1] Oligonucleotides were quantified spectrophotometrically at a wavelength of 260 nm.

General Hybridization Procedure

The hybridization was carried out by dissolving ss DNA-*b*-PPO diblock copolymer and the complementary strand or the long ss DNA templates, T110 and T88, in TAE buffer (20 mM tris(hydroxymethyl)aminomethane-HCl, pH 8.0; 10 mM acetic acid, 0,5 mM EDTA) containing Na^+ (100 mM) and Mg^{2+} (60 mM). The mixture was heated to 95°C and was slowly cooled to room temperature over the course of 3 days (1 degree per hour) by using a Biometra polymerase chain reaction (PCR) thermocycler (Biometra GmbH, Germany). The final concentration of DNA was between 2-5 µM.

Material Preparation for FCS Experiments

ss DNA-*b*-PPO: Ss DNA-*b*-PPO micelles were hybridized with the complementary sequence which was functionalized with Alexa488 (Invitrogen, USA) at the 5' end. The ratio of ss DNA-*b*-PPO to ODN carrying the dye was adjusted to be 1 % so that the predominant form of DNA within the corona remains single stranded.

Ds DNA-*b*-PPO: ss DNA-*b*-PPO was first hybridized with the dye as described above, then they were completely hybridized with the complementary sequence to obtain double stranded micelles.

DNA-*b*-PPO-T110: ss DNA-*b*-PPO was hybridized with equimolar amounts of Cy3 modified T110. The final dye concentration was 1 µM.

DNA-*b*-PPO-T88: ss DNA-*b*-PPO was hybridized with equimolar amounts of Cy3 modified T88. The final dye concentration was 1 µM.

DNA Sequences:

ss DNA-*b*-PPO: 5'-CCTCGCTCTGCTAATCCTGTTA-3'

Complementary: 5'-TAACAGGATTAGCAGAGCGAGG-3'

T110 : 5'- $(TAACAGGATTAGCAGAGCGAGG)_5$-3'

T88 : 5'- $(TAACAGGATTAGCAGAGCGAGG)_4$-3'

II. Fluorescence correlation spectroscopy (FCS)

FCS measurements were carried out on a confocal setup of local design based on an Olympus IX71 inverted microscope. The 488 nm line of an argon ion laser (model 2020, Spectra Physics) was attenuated to 150 µW before focusing into the buffer solution by a water immersion objective (40 x, N.A. 1.15, Olympus). The solution was placed on a microscope coverslide as a droplet of 25 to 50 µl. Scattered laser light was blocked by a dichroic beam splitter (DCXR 488, AHF, Tübingen, Germany), and fluorescence was collected in the spectral range from 532 to 570 nm using interference filters (AHF). Single photons were detected by an avalanche photodiode (SPCM AQR-14, Perkin Elmer) and registered by a TCSPC device (PC card SPC-630, Becker & Hickl, Berlin, Germany) for software calculation of the autocorrelation functions, or by a real time hardware correlator (PC card ALV-5000 E, ALV, Langen, Germany).

The fluorescence intensity autocorrelation functions, $G(\tau_c)$, were fitted with a single diffusion time, τ_D, for the sample according to

$$G(\tau_c) = 1/N_f\,[1/(1 + \tau_c/\tau_D)]\,[1/(1 + (\omega/z)^2(\tau_c/\tau_D))]^{1/2}[1 - T + T\exp(-\tau_c/\tau_T)] \quad (1)$$

with N_F, average number of fluorescent molecules in the confocal detection volume, τ_c, correlation time, ω/z, the ratio of the $1/e^2$ radii of the detection volume in radial and axial directions, T, average fraction of fluorophores in the triplet state, and τ_T, lifetime of the triplett state of the fluorophore. The ω/z was measured with a R6G solution as the reference and was kept fixed at this value during the subsequent fitting of the autocorrelation functions of the DNA-PPO micelle solutions.

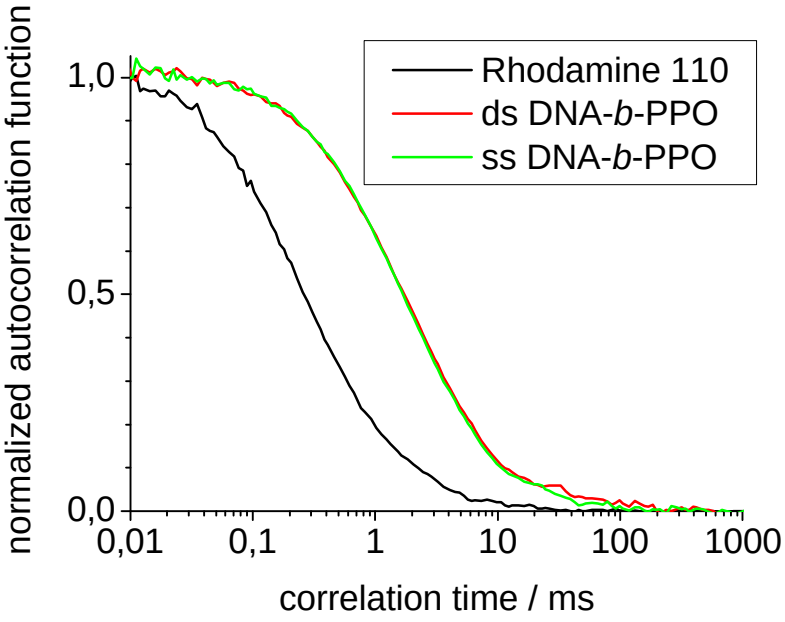
The diffusion coefficient, D, is related to the diffusion time by

$$\tau_D = \omega^2 / 4D \quad (2)$$

and to the frictional coefficient, f_{sphere}, of a sphere with radius R_0 by

$$f_{sphere} = kT / D = 6\pi\eta R_0 \quad (3)$$

which allows for the calculation of the radii of the spherical micelles.



Supporting Figure 1: Normalized autocorrelation functions of the DNA-*b*-PPO micelles in buffer solutions with an ss DNA corona (green curve), and with a ds DNA shell (red curve). As a reference Rhodamine 110 in water (black curve) was measured.

Extrapolation of the diffusion times from the rod-like structures measured by AFM

The parallel-aligned dimers of the DNA-PPO hybrids on the T110 template can be treated as a cylinder of length 2*a* and radius *b*. The volume, V_{dimer}, of the rod is

$$V_{rod} = 2\pi\, a\, b^2 \qquad (4)$$

which corresponds to a hypothetical spherical volume with an apparent radius, R_0,

$$R_0 = (1.5\, a\, b^2)^{1/3} \qquad (5)$$

The axial ratio of length and radius of the cylinder, P, is:

$$P = a / b \quad (6)$$

The frictional coefficient f_{rod} of the cylinder is related to the apparent radius R_0 and the axial ratio P by

$$f_{rod} = 6 \pi \eta R_0 [(2/3)^{1/3} P^{2/3}]/[\ln (2P) - 0.30] \quad (7)$$

with η, viscosity of the solvent.

The frictional coefficient is related to the diffusion time τ_D combining (2) and (3) to

$$f_{rod} = \tau_D (4 kT / \omega^2) \quad (8)$$

with ω, radial $1/e^2$ radius of the detection volume in the FCS measurements.

Accordingly the expected ratio of the diffusion times for the aggregates of the hybridization products DNA-*b*-PPO-T110 to ds T110 was calculated using the AFM structural information.

For the DNA-*b*-PPO-T110 the length of the rod resulted in *a* = 14.55 nm, a mean radius of *b* = 2.3 nm and an axial ratio of P = 6.3 which yielded $V''_{rod} = \pi \cdot 154\ nm^3$ and $R_o'' = 4.87$ nm. The frictional coefficient was $f'' = 6\pi\eta\ 4.87\ nm \cdot 1.34 = 6\pi\eta \cdot 6.5$ nm.

For the controls we used a = 18.7 nm, b = 0.975 nm and P = 19 yielding $V'_{rod} = \pi \cdot 36\ nm^3$ and $R_o' = 2.99$ nm. The frictional coefficient was calculated to $f' = 6\pi\eta\ 2.99\ nm\ 1.87 = 6\pi\eta \cdot 5.59$ nm.

The relative diffusion time changes predicted from the AFM structure resulted in a factor $\tau_{D,\ Dimer}/\tau_{D,\ controls} = 1.16$ for the T110-associated DNA-PPO and for the T110 controls.

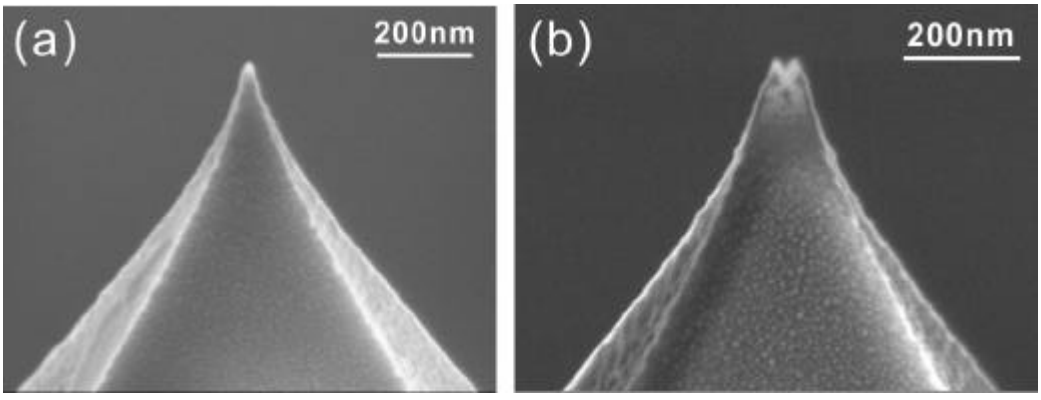
However, if we assume that the dimeric rods would have a doubled hydrodynamic volume, the expected ratio of the diffusion times should be $\tau_{D,\ Dimer} / \tau_{D,\ controls} = 1.26$ which is also in good agreement with the FCS data. To match the measured diffusion time ratio of 1.29, we have to consider an aspect ratio of P = 5.1 for the hydrated dimer, which yields $\tau_{D,\ Dimer} / \tau_{D,\ controls} = 1.288$ corresponding to an apparent radius of 2.85 nm for the dimer. This could also result from a higher aggregate, i.e. a trimer or tetramer, in solution.

III. SFM Measurements

AFM imaging of DNA block copolymers in buffer solution:

A drop of 20 µL block copolymer buffer solution (10 mM Tris-HCl pH 7.4, 1 mM $NiCl_2$) was deposited on freshly cleaved mica (Plano GmbH, Germany) and left to incubation for 5 min. Then the surface was washed with 200 µL buffer solution and mounted onto a piezoelectric E-scanner (Veeco Instruments, California). In particular we ensured that the sample was always kept wet during the sample handling. Imaging was performed under tapping mode AFM in a liquid cell on a Multimode Nanoscope IIIa (Veeco Instruments, California USA). Oxide-sharpened silicon nitride cantilevers (NP-S, Veeco Instruments, California; 115 µm long, 17 µm wide, 0.6 µnm thick) with an integrated tip (a spring constant of 0.32 N/m and a resonance frequency of 56 kHz in air) were applied. A driving frequency between 8 – 10 kHz for imaging was selected in existence of buffer solution. The images (512x512 pixels) were recorded with a scan size of 500 x 500 nm^2 and 1 x 1 μm^2 at a scan rate of 1 Hz and by adjusting soft tapping mode.[2] The raw topography data has been modified by applying the first order "flatten" filter. The maximum height of aggregates was calculated by means of local roughness analysis.

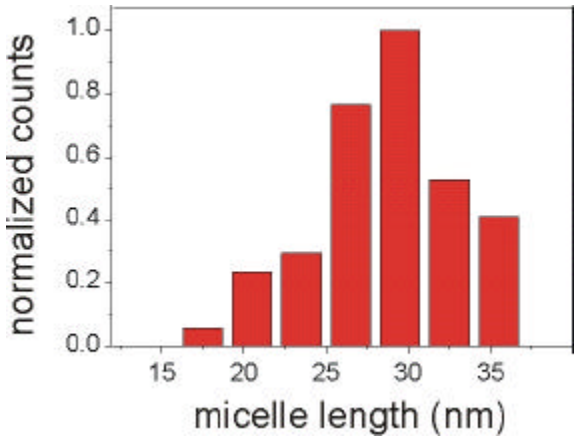
The tip radii were measured by scanning electron microscopy (SEM) after having performed the SFM measurements. For the images presented and used for analysis we determined tip radii of curvatures < 20 nm (Supporting Figure 2a). In some cases double tips have been found (Supporting Figure 2b). These tips can produce imaging artifacts appearing as double structures in the topography. Therefore all measurements where we found double tips were not considered. In addition, we can exclude artifacts from a double tip since the appearing aggregates show different orientation relative to the scanning direction in one image.



Supporting Figure 2: The SEM image of the tip **(a)** with a radius of curvature < 20 nm, **(b)** showing a double-tip.

SFM length measurements of the rod like micelles

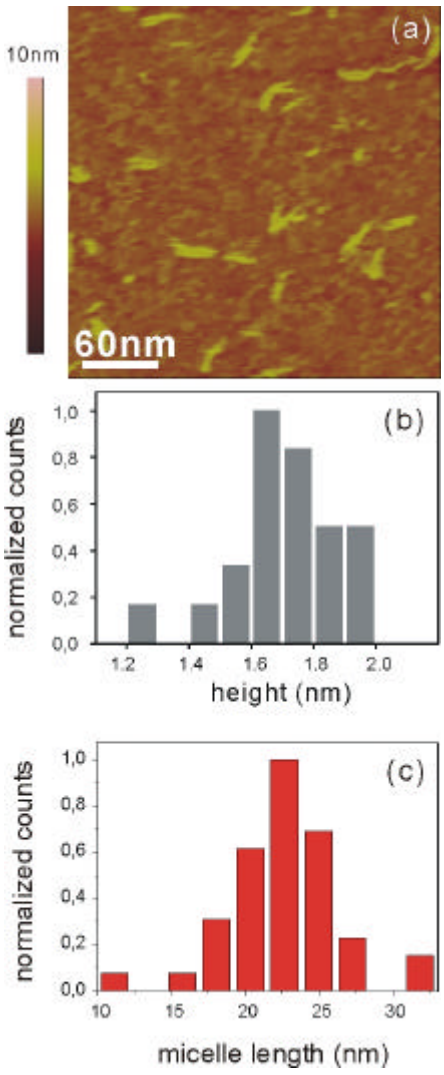
Length measurements of molecules are influenced by the SFM-tip size. We have considered the SFM-tip size effect by measuring in each picture the diameters of isolated ds DNA strands. The diameters were taken as the full width at half maximum (FWHM) of a line section across the DNA molecule. Typically we measured values between 4 and 6 nm for the T110 and T88 structures. Since the ds-DNA has a diameter of 2 nm, we consider an error owing to the tip shape SFM_{error} between 2 and 4 nm. This additive effect was then considered in the lengths measurements as well. The lengths were measured by poly line profiles along the ds-DNA molecules using SPIP software. The poly-line was taken since we can collect also the length data of partially curved ds-DNA molecules. We have taken the length between points where the height is decreased by one half of the average height of the molecule (L_{SFM}). Assuming the same error as found in the estimation of the diameter in the same picture, the lengths of the ds-DNA (L_{DNA}) molecules is taken as $L_{DNA} = L_{SFM} - SFM_{error}$. The measured values are plotted in histograms shown in supporting Figure 6. Then Gaussian distributions were fitted to the histograms. The center values of the Gaussian distribution together with the error are reported. For T110 we found a lengths $L_{DNA,T110} = 29.1 \pm 6.5$ nm (Supporting Figure 3).



Supporting Figure 3: The corresponding histogram of the length determination of T110/DNA-*b*-PPO hybridization products.

SFM analysis of rod-like micelles composed of ss DNA-*b*-PPO and template T88

The AFM study was performed under 25 ng/µL in buffer. Similar to T110, they formed rod like structures on mica surfaces (Supporting Figure 4a).



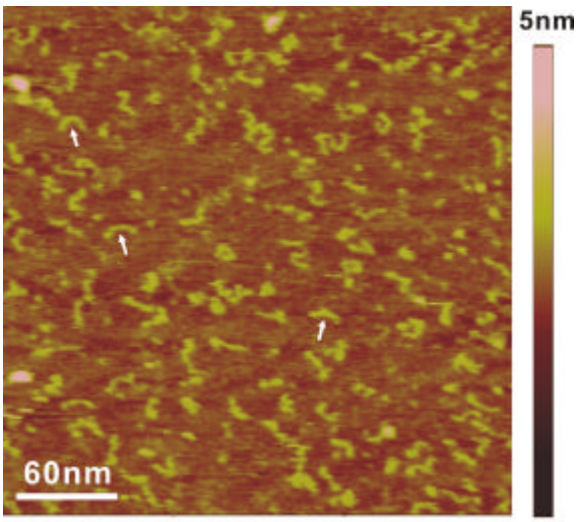
Supporting Figure 4: a) SFM topography image of the hybridization products of DNA-*b*-PPO and T88. b) The height of the rod-like aggregates was expressed in a histogram. c) The corresponding histogram of the length of T88/DNA-*b*-PPO micelles.

The histogram shows a similar height distribution of the rod-like aggregates compared to rods composed of T110 and DNA-*b*-PPO (Supporting Figure 4b). From the Gaussian distribution we determined a length $L_{DNA,T88}$ of 22.7 ± 5.1 nm (Supporting Figure 4c).

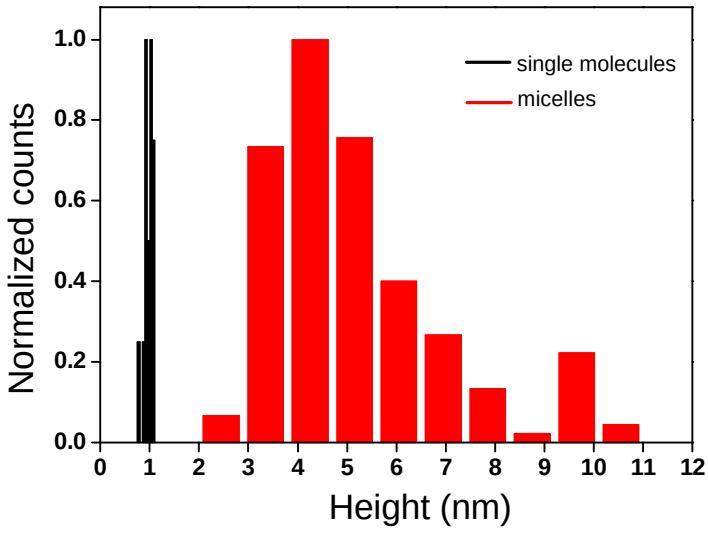
Single molecules of ss DNA-*b*-PPO below the CMC

Single molecules should appear at a concentration below the critical micelle concentration (CMC). Measurements by means of scanning force microscopy at a concentration of 1 ng/µL reveal a completely different type of surface topography (Supporting Figure 5). In this micrograph single molecules are visualized which in some cases show the

formation of dimers (see arrows) owing to the hydrophobic interaction of PPOs. The contour lengths and the height of the molecules are consistent with the expected molecular dimensions. Below the CMC, the observed molecular structure is significantly different from the observed rod-like micelles and spherical micelles reported in the manuscript. At concentrations below CMC we have not observed micellar structures. This is reflected by the comparison of height histograms obtained from measurements above and below CMC (Supporting Figure 6). We find a mean height around 1 nm for single molecules below CMC (black) and a mean height value around 4 – 6 nm for the micelles (above CMC, red).



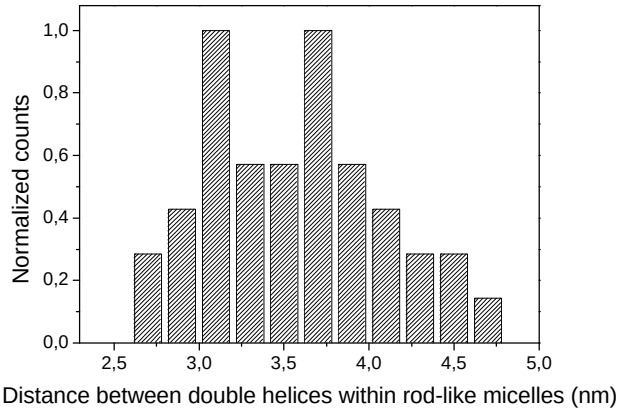
Supporting Figure 5: SFM topography image of the ssDNA-*b*-PPO below the CMC.



Supporting Figure 6: The height histograms of the single molecules of DNA-*b*-PPO (black) and the ss spherical micelles of DNA-*b*-PPO (red).

Distance between double helices within rod-like micelles

To determine the distance between the two double helices within rod-like micelles fabricated with T110 we have measured the peak-to-peak distance at different positions along the aggregate (Supporting Figure 7). The histogram shows that the two ds DNA strands are typically separated by 3 – 4 nm.



Supporting Figure 7: The histogram of the distance between the two double helices within rod-like micelles fabricated with T110 and DNA-*b*-PPO.

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[2] S.N. Magonov, *Encyclopedia of Analytical Chemistry*, R.A. Meyers (Ed.) John Wiley & Sons Ltd, Chichester, 2000, pp. 7432–7491.